Figure 1A

Mutagenize Rgene by error-prone PCR

↓

Ligate Rgene* to expression vector

↓

Transform into ER2502 [pMgene]

↓

Select survivors at 25°C-42°C on LB agar + Amp, Cam, IPTG

↓

Make a plasmid library of pRgene* and pMgene

↓

Eliminate pMgene by digestion, but leave pRgene* intact

↓

Transform ER1992 *dinD::lacZ* indicator strain with pRgene* library

↓

Select individual dark blue colonies at 30°C-42°C on LB agar + X-gal, Amp (No IPTG)

↓

Prepare plasmid DNA from individual dark blue colonies

↓

Transform pRgene* clones into ER1992 [pMgene]

↓

Select **white** colonies at 30°C-42°C on LB agar + X-gal, Amp, Cam

↓

Screen white colonies

The white colonies may contain Rgene* variants with desired specificity

Figure 1B

**Genetic Selection of *Bst*YI Variants with 5'-AGATCT-3' specificity**

Mutagenize *bstYIR* gene by error-prone PCR

↓

Ligate *bstYIR** to pAGR3 (Ptac)

↓

Transform into ER2502 [pSYX-BglIIM]

↓

Select survivors at 37°C on LB agar + Amp, Tet, 0.3 mM IPTG

↓

Make a plasmid library of pAGR3-BstYIR* and pSYX-BglIIM

↓

Eliminate pSYX-BglIIM by digestion, but leave pAGR3-BstYIR* intact

↓

Transform ER1992 *dinD::lacZ* indicator strain with pAGR3-BstYIR* library

↓

Select individual dark blue colonies at 37°C on LB agar + X-gal, Amp (No IPTG)

↓

Prepare plasmid DNA from individual dark blue colonies

↓

Transform pAGR3-BstYIR* clones into ER1992 [pSYX-BglIIM]

↓

Select **white** colonies at 37°C on LB agar + X-gal, Amp, Tet

↓

Screen white colonies

The white colonies may contain *Bst*YI* mutants with *Bgl*II specificity

FIGURE 2

```
     ATGAGAATTGTTGAAGTATATATTCGCATTTGAACGGGTTGGAATACATACAAGTTCACTTG
1    ---------+---------+---------+---------+---------+---------+ 60
      M  R  I  V  E  V  Y  S  H  L  N  G  L  E  Y  I  Q  V  H  L
     CCACATATTTGGGAAGAAATTCAAGAAATTATTGTTTCTATTGACGCAGAAGCTTGTAGA
61   ---------+---------+---------+---------+---------+---------+ 120
      P  H  I  W  E  E  I  Q  E  I  I  V  S  I  D  A  E  A  C  R
     ACGAAGGAATCAAAAGAAAAGACAAAACAAGGACAAATACTTTATAGTCCCGTAGCTTTA
121  ---------+---------+---------+---------+---------+---------+ 180
      T  K  E  S  K  E  K  T  K  Q  G  Q  I  L  Y  S  P  V  A  L
     AATGAAGCATTCAAGGAAAAATTAGAAGCAAAAGGTTGGAAAGAAAGTCGAACAAACTAT
181  ---------+---------+---------+---------+---------+---------+ 240
      N  E  A  F  K  E  K  L  E  A  K  G  W  K  E  S  R  T  N  Y
     TATGTGACTGCTGACCCAAAGCTGATTCGTGAAACATTATCACTTGAACCAGAGGAACAA
241  ---------+---------+---------+---------+---------+---------+ 300
      Y  V  T  A  D  P  K  L  I  R  E  T  L  S  L  E  P  E  E  Q
     AAGAAAGTGATTGAAGCCGCAGGAAAAGAAGCATTAAAGTCTTATAATCAAACGGATTTT
301  ---------+---------+---------+---------+---------+---------+ 360
      K  K  V  I  E  A  A  G  K  E  A  L  K  S  Y  N  Q  T  D  F
     GTAAAAGATAGAGTGGCAATAGAAGTTCAATTCGGAAAATATTCTTTTGTCGCTTATGAC
361  ---------+---------+---------+---------+---------+---------+ 420
      V  K  D  R  V  A  I  E  V  Q  F  G  K  Y  S  F  V  A  Y  D
     CTTTTCGTCAAACACATGGCTTTCTATGTTAGTGATAAAATTGACGTTGGTGTCGAAATA
421  ---------+---------+---------+---------+---------+---------+ 480
      L  F  V  K  H  M  A  F  Y  V  S  D  K  I  D  V  G  V  E  I
     TTGCCAATGAAGGAATTATCAAAAGAAATGTCTTCGGGAATCAGTTATTACGAAGGTGAA
481  ---------+---------+---------+---------+---------+---------+ 540
      L  P  M  K  E  L  S  K  E  M  S  S  G  I  S  Y  Y  E  G  E
     TTATACAATGTGATACGGCAAGGTCGTGGCGTTCCTGCCGTTCCGTTGGTTTTAATCGGG
541  ---------+---------+---------+---------+---------+---------+ 600
      L  Y  N  V  I  R  Q  G  R  G  V  P  A  V  P  L  V  L  I  G
     ATTGCCCCTTAA
601  ---------+-- 612
      I  A  P  *
```
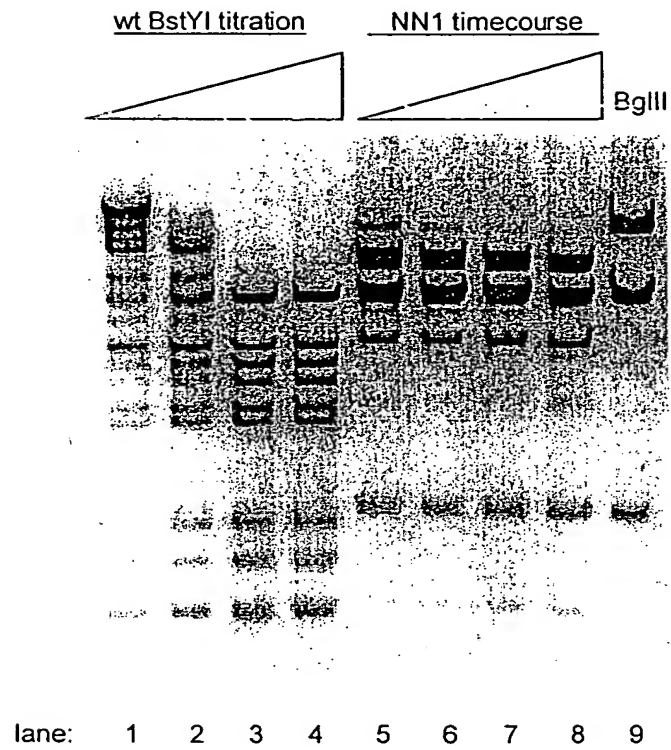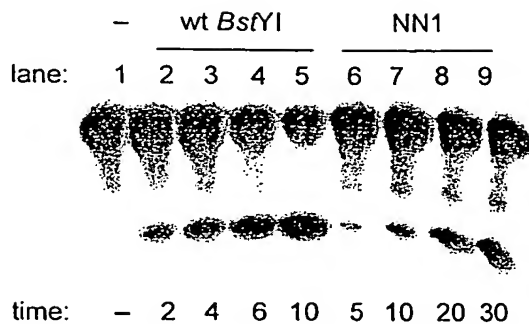
Figure 3
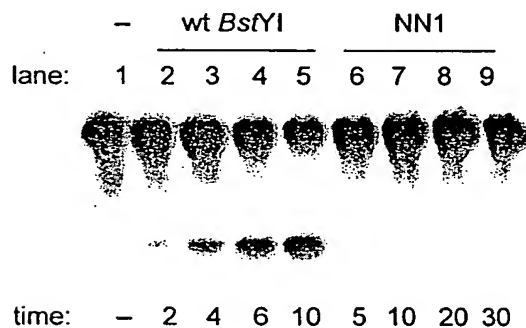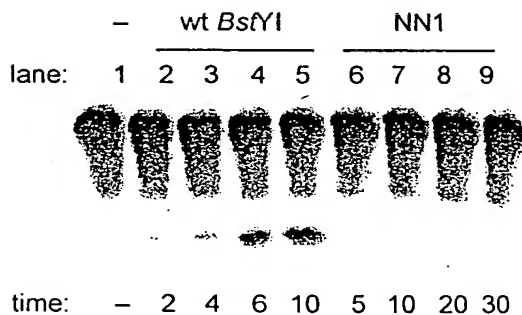
FIGURE 4A

5'.....AGATCT.....3'
3'.....TCTAGA.....5'

FIGURE 4B

5'.....GGATCC.....3'
3'.....CCTAGG.....5'

FIGURE 4C

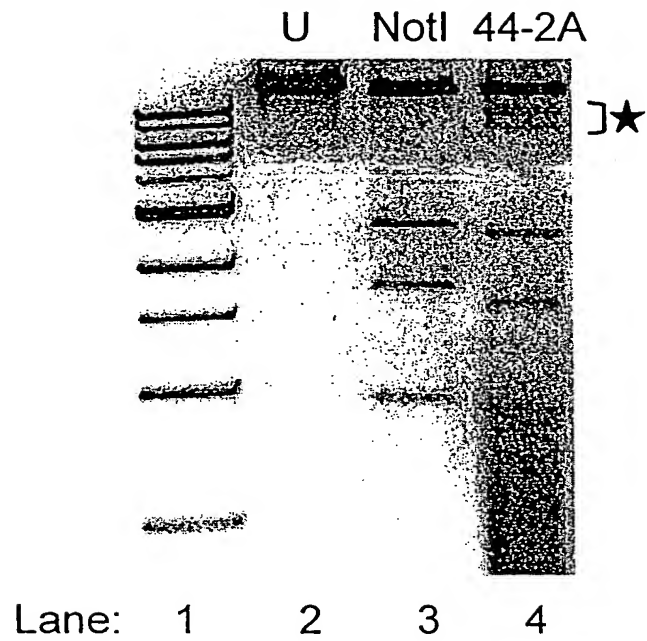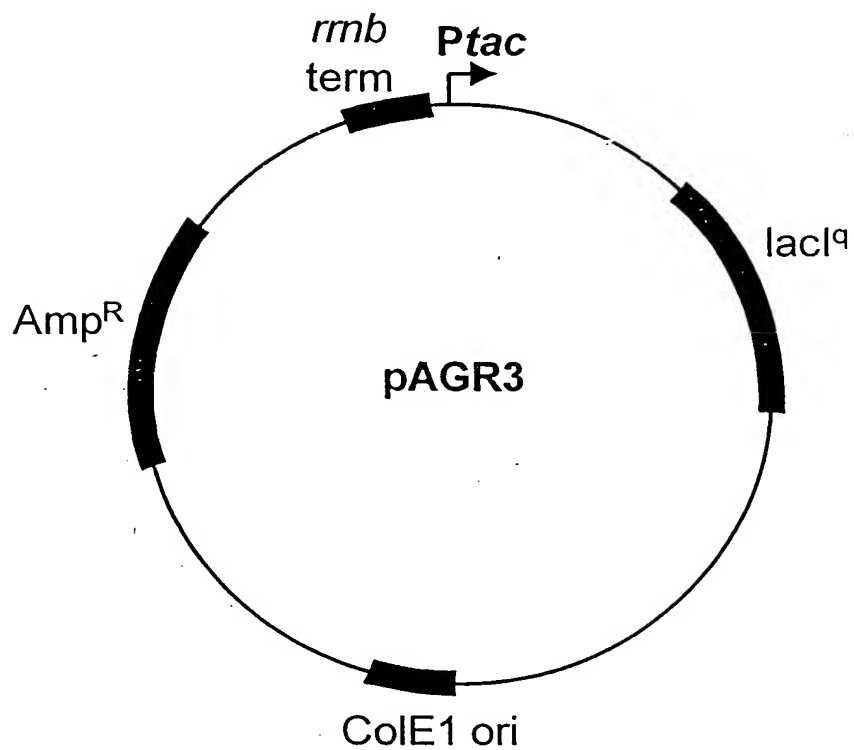5'.....AGATCC.....3'
3'.....TCTAGG.....5'

Figure 5

U   NotI 44-2A

]★

Lane: 1   2   3   4

Figure 6

Figure 7